

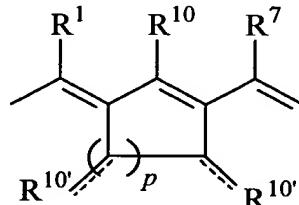
We Claim:

1. A mobility-modifying cyanine dye comprising: (i) a first substituted or unsubstituted parent heteroaromatic benzazole/benzazolium ring system having a linking moiety of the formula $-L-LG$, where L is a linker and LG is a linking group, attached to the heteroaromatic ring nitrogen; (ii) a second substituted or unsubstituted parent heteroaromatic benzazole/benzazolium ring system having a mobility-modifying moiety attached to the heteroaromatic ring nitrogen; and an electron delocalizing bridge connecting the first and second parent benzazole/benzazolium rings *via* their respective C_2 carbons, wherein when said mobility-modifying moiety has a net charge of -2 or less or +1 or greater.

2. The mobility-modifying cyanine dye of Claim 1 in which the bridge is a compound selected from the group consisting of:

(B.1) $-(CR^1=CR^2)_k-(CR^3=CR^4)_l-(CR^5=CR^6)_m-CR^7=$
and

(B.2)



wherein:

k , l , and m are each independently integers from 0 to 1;

R^1 , R^2 , R^3 , R^4 , R^5 , R^6 and R^7 are each independently selected from the group consisting of hydrogen, halogen, $-F$, $-Cl$, $-CN$, $-CF_3$, (C_1-C_6) alkyl, (C_5-C_{14}) aryl or 5-14 membered heteroaryl;

R^{10} and $R^{10'}$ are each independently selected from the group consisting of hydrogen, oxygen, halogen, $-F$, $-Cl$, $-CN$, $-CF_3$, $-OR$, $-SR$, $-NRR$, (C_1-C_6) alkyl, (C_5-C_{14})

aryl or 5-14 membered heteroaryl, where each R is independently hydrogen or (C₁-C₆) alkyl; and

5 *p* is an integer from 0 to 2, where in structural formula (B.2), the dotted lines at substituents R¹⁰ represent bonds that may be independently either single bonds or a double bonds, depending upon the identities of the substituents.

10 3. The mobility-modifying cyanine dye of Claim 2 in which the bridge is a compound of structural formula (B.1).

15 4. The mobility-modifying cyanine dye of Claim 3 in which the sum of *k*, *l* and *m* is 2.

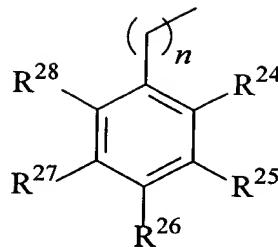
20 5. The mobility-modifying cyanine dye of Claim 2 in which the bridge is a compound of structural formula (B.1) wherein R¹, R², R³, R⁴, R⁵, R⁶ and R⁷ are each hydrogen.

25 6. The mobility-modifying cyanine dye of Claim 1 in which the bridge is -CH=CH-CH=CH-CH=.

7. The mobility-modifying cyanine dye of Claim 1 in which the mobility-modifying moiety has a net positive charge.

8. The mobility-modifying cyanine dye of Claim 1 in which the mobility-modifying moiety has a net negative charge.

9. The mobility-modifying cyanine dye of Claim 1 in which the mobility-modifying moiety has the structure:



or a salt thereof, wherein:

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n is an integer from 1 to 6 (preferably 1 to 3);

10 R^{24} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$; or when taken together with R^{25} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups;

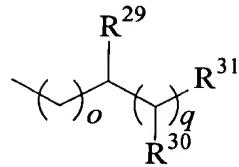
15 R^{25} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$, or when taken together with R^{24} or R^{26} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups;

20 R^{26} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$, or when taken together with R^{25} or R^{27} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups; and

25 R^{27} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$, or when taken together with R^{26} or R^{28} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups; and

25 R^{28} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$, or when taken together with R^{27} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups.

10. The mobility-modifying cyanine dye of Claim 1 in which the mobility-modifying moiety has the structure:



or a salt thereof, wherein:

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o is an integer from 1 to 3;

q is an integer from 1 to 3;

R²⁹ is a strong anionic substituent, -S(O)₂O⁻ or -O-S(O)₂O⁻,

each R³⁰ is independently selected from the group consisting of hydrogen a strong anionic substituent, -S(O)₂O⁻ and -O-S(O)₂O⁻; and

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R³¹ is selected from the group consisting of hydrogen, a strong anionic substituent, -S(O)₂O⁻, -O-S(O)₂O⁻ and -CH₃,

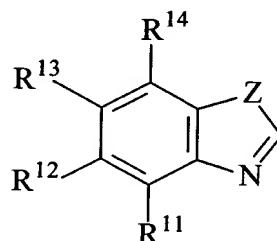
with the proviso that MM has a net charge of at least -2 at a pH in the range of about pH 6 to pH 10.

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11. The mobility-modifying cyanine dye of Claim 1 in which the first and second heteroaromatic benzazole/benzazolium ring systems are the same.

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12. The mobility-modifying cyanine dye of Claim 1 in which the first parent heteroaromatic benzazole/benzazolium ring system has the structure:



or a salt thereof, wherein:

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Z is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹-, where R⁸ and R⁹ when taken alone, are each independently (C₁-C₆) alkyl, or when taken together

are (C₄-C₅) alkylene or (C₄-C₅) alkano;

5 R¹¹, R¹², R¹³ and R¹⁴, when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

10 or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) arylene, (C₆-C₁₀) arylene independently substituted with one or more W, 6-10 membered heteroarylene and 6-10 membered heteroarylene independently substituted with one or more W; and

15 each W is independently -R, -X, =O, -OR, =S, -SR, -NRR, =NR, (C₁-C₆) perhaloalkyl, -CX₃, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -NHOH, -S(O)₂R, -C(O)R, -C(S)R, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', and -C(NR)NRR, wherein:

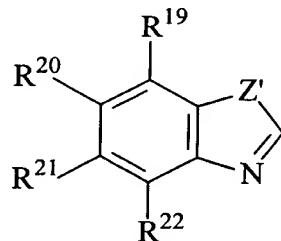
20 each X is independently a halogen;

25 each R is independently -H, -NR"R", -C(O)R", -S(O)₂R", (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

30 each R' is independently (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl and (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each R" is independently -H, (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl.

13. The mobility-modifying cyanine dye of Claim 1 in which the second parent heteroaromatic benzazole/benzazolium ring system has the structure:

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or a salt thereof, wherein:

10 Z' is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹, where R⁸ and R⁹, when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkylene or (C₄-C₅) alkano;

15 R¹⁹, R²⁰, R²¹ and R²², when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

20 or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) arylene, (C₆-C₁₀) arylene independently substituted with one or more W, 6-10 membered heteroarylene and 6-10 membered heteroarylene independently substituted with one or more W; and

25 each W is independently -R, -X, =O, -OR, =S, -SR, -NRR, =NR, (C₁-C₆)

perhaloalkyl, -CX₃, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -NHOH, -S(O)₂R, -C(O)R, -C(S)R, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', and -C(NR)NRR, wherein:

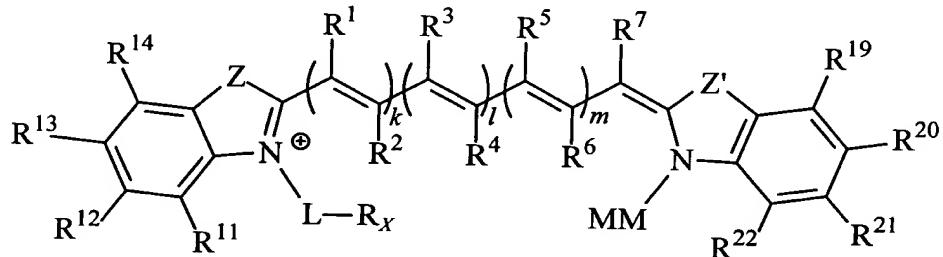
each X is independently a halogen;

5 each R is independently -H, -NR"R", -C(O)R", -S(O)₂R", (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

10 each R' is independently (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl and (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each R" is independently -H, (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl.

14. A mobility-modifying cyanine dye according to Claim 1 in which the first and second heteroaromatic benzazole/benzazolium rings are each the same or different substituted or unsubstituted indoline/indolinium ring.

15. The mobility-modifying cyanine dye of Claim 1 which has the structure:



20 or a salt thereof, wherein:

k, l, and m are each independently integers from 0 to 1;

25 R¹, R², R³, R⁴, R⁵, R⁶ and R⁷ are each independently selected from the group consisting of hydrogen, halogen, -F, -Cl, -CN, -CF₃, (C₁-C₆) alkyl, (C₅-C₁₄) aryl and 5-14 membered heteroaryl;

MM is a mobility-modifying moiety;

L is a linker;

R_x is a reactive functional group;

5 Z is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹-, where R⁸ and R⁹ when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkylene or (C₄-C₅) alkano;

10 Z' is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹, where R⁸ and R⁹, when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkylene or (C₄-C₅) alkano;

15 R¹¹, R¹², R¹³ and R¹⁴, when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

20 or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) arylene, (C₆-C₁₀) arylene independently substituted with one or more W, 6-10 membered heteroarylene and 6-10 membered heteroarylene independently substituted with one or more W;

25 R¹⁹, R²⁰, R²¹ and R²², when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl

independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

5 or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) arylene, (C₆-C₁₀) arylene independently substituted with one or more W, 6-10 membered heteroarylene and 6-10 membered heteroarylene independently substituted with one or more W;

10 each W is independently -R, -X, =O, -OR, =S, -SR, -NRR, =NR, (C₁-C₆) perhaloalkyl, -CX₃, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -NHOH, -S(O)₂R, -C(O)R, -C(S)R, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', and -C(NR)NRR, wherein:

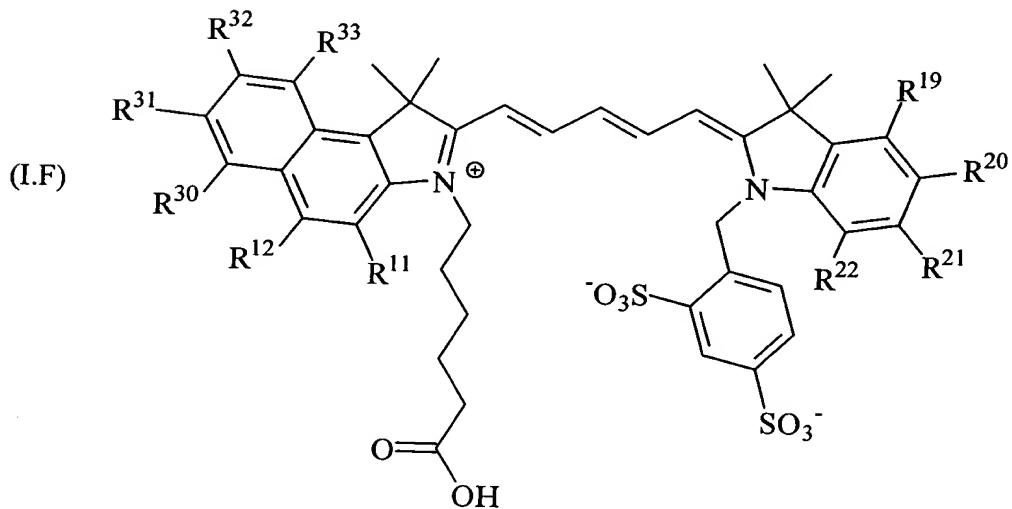
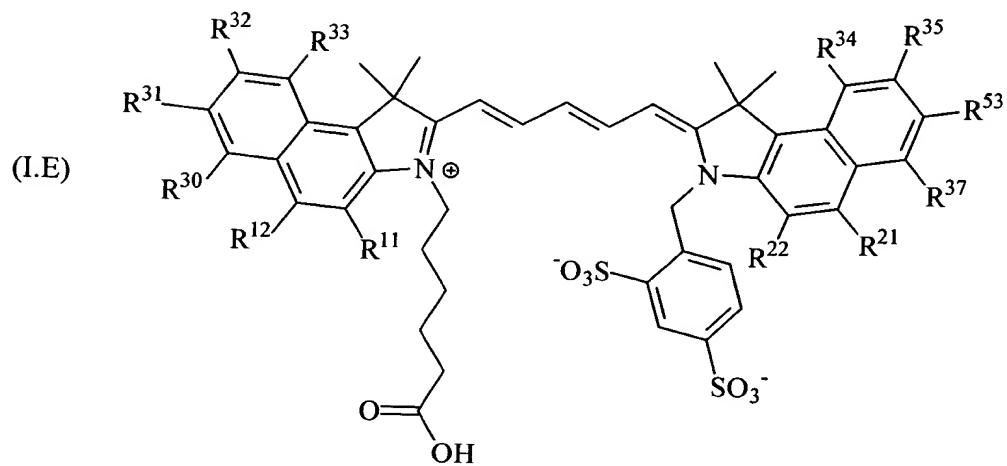
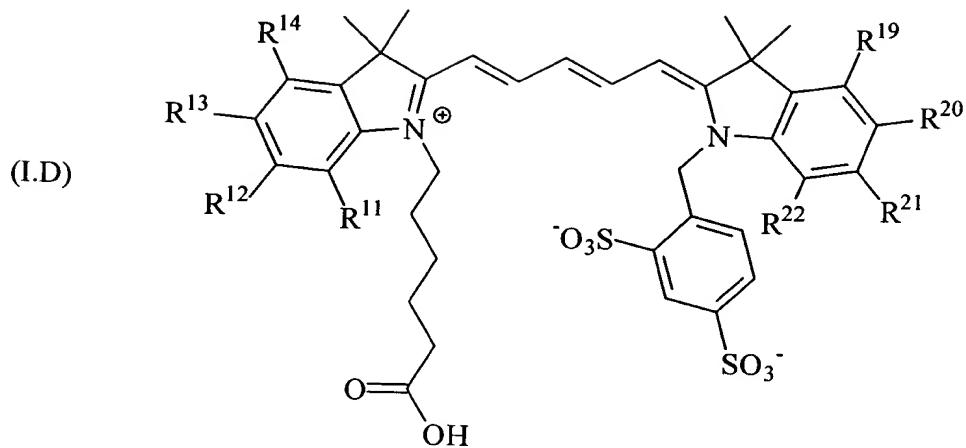
15 each X is independently a halogen;

each R is independently -H, -NR"R", -C(O)R", -S(O₂)R", (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

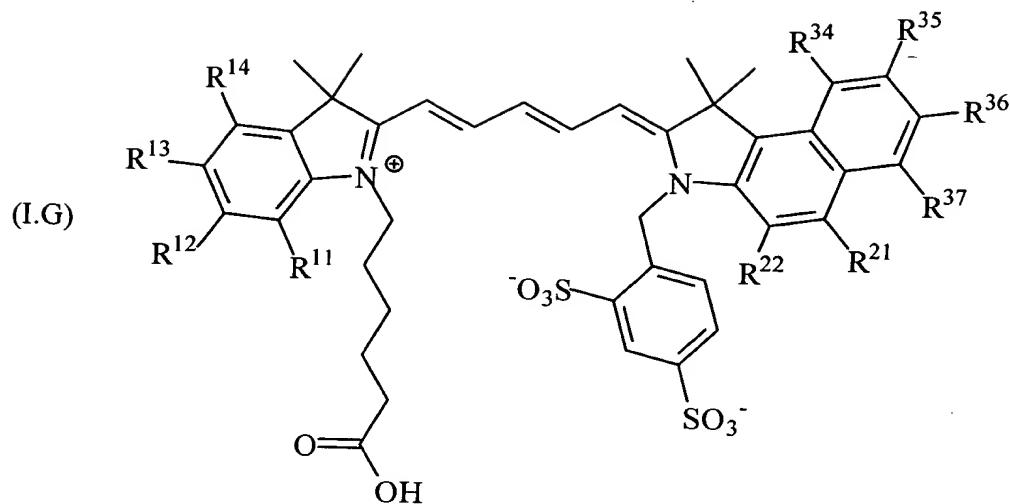
20 each R' is independently (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl and (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each R" is independently -H, (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl.

25 16. The mobility-modifying cyanine dye of Claim 15 in which Z is -NR⁸R⁹-, where R⁸ and R⁹ are each independently (C₁-C₆) alkano; and Z' is -NR⁸R⁹-, where R⁸ and R⁹ are each independently (C₁-C₆) alkano.

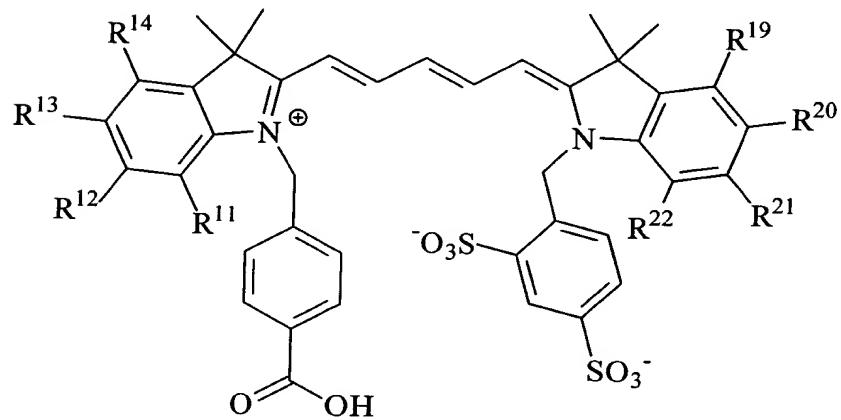
30 17. The mobility-modifying cyanine dye of Claim 15 which is selected from the group consisting of:



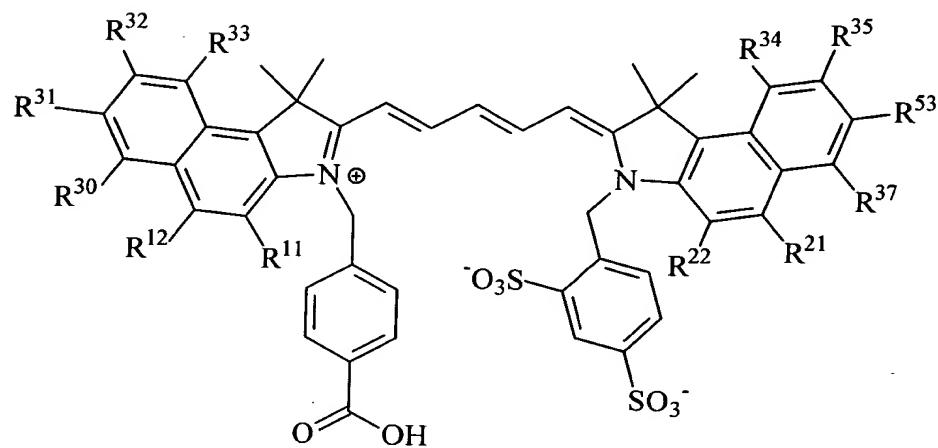
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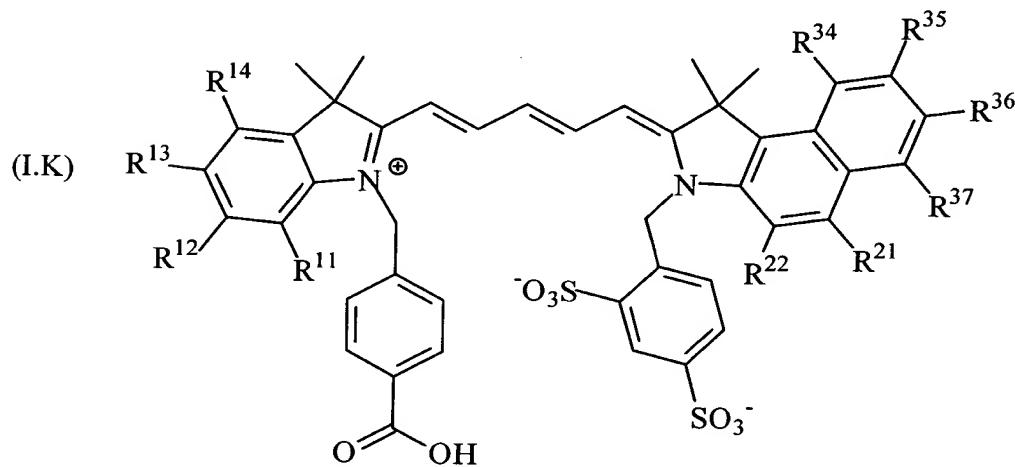
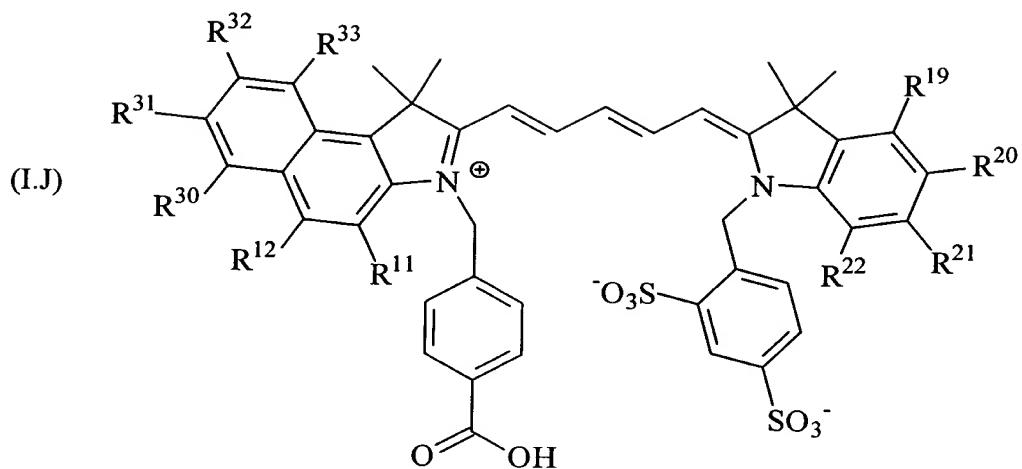


(I.H)



(I.I)



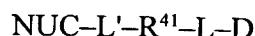


or a salt thereof, wherein:

R¹¹, R¹², R¹³, R¹⁴, R¹⁹, R²⁰, R²¹, R²², R³⁰, R³¹, R³², R³³, R³⁴, R³⁵, R³⁶ and R³⁷

are each independently selected from the group consisting of hydrogen, -S(O)₂O⁻ and -O-S(O)₂O⁻.

15 18. A labeled nucleoside/tide or nucleoside/tide analog having the structure:



20 or a salt thereof, wherein:

D is a mobility-modifying cyanine dye chromophore;

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L is a first linker which is attached to D at a heteroaromatic ring nitrogen;
R⁴¹ is a covalent linkage;
NUC is a nucleoside/tide or nucleoside/tide analog; and
L' is a second linker which is attached to the nucleobase or sugar moiety of
NUC.

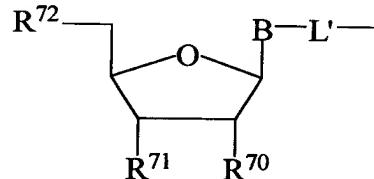
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19. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 which is enzymatically incorporable.

20. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 which is a terminator.

21. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 which is enzymatically extendable.

22. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 in which -L'-NUC taken together has the structure:



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L is a first linker which is attached to D at a heteroaromatic ring nitrogen;
R⁴¹ is a covalent linkage;

NUC is a nucleoside/tide or nucleoside/tide analog; and

L' is a second linker which is attached to the nucleobase or sugar moiety of

NUC.

B is a nucleobase;

L' is (C₁-C₂₀) alkyldiyl, (C₁-C₂₀) alkylene, (C₂-C₂₀) alkyno, (C₂-C₂₀) alkeno

25

2-20 membered heteroalkyldiyl, 2-20 membered heteroalkylene, 2-20 membered

heteroalkyno or 2-20 membered heteroalkeno;

R⁷⁰ and R⁷¹, when taken alone, are each independently selected from the

group consisting of hydrogen, hydroxyl and a moiety which blocks polymerase-mediated

template-directed polymerization, or when taken together form a bond such that the

illustrated sugar is 2',3'-didehydroribose; and

R⁷² is selected from the group consisting of hydroxyl, a phosphate ester

having the formula —O—P(=O)(O⁻)_a—O—P(=O)(O⁻)_a—OH, where *a* is an integer from 0 to 2 and a

phosphate ester analog.

5

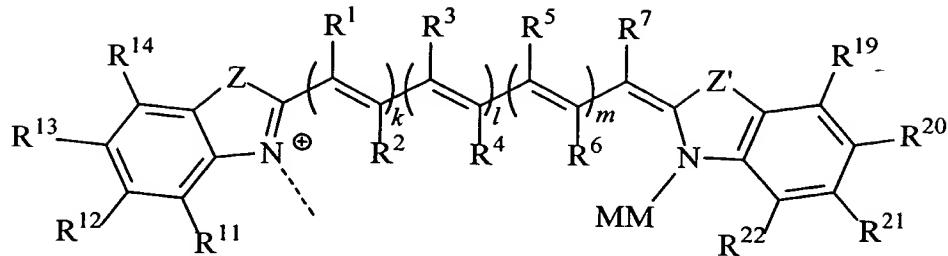
23. The labeled nucleoside/tide or nucleoside/tide analog of Claim 22 in which L' is selected from the group consisting of:

—C≡C—CH₂—, where the terminal *sp* carbon is covalently attached to nucleobase B and the terminal methylene (*sp*³) carbon is covalently attached to R⁴¹; and
—C≡C—CH₂—O—CH₂—CH₂—NR⁴⁷—R⁴⁸—, where R⁴⁷ is hydrogen or (C₁—C₆) alkyl and R⁴⁸ is —C(O)—(CH₂)_r—, —C(O)—CHR⁴⁹—, —C(O)—C≡C—CH₂— or —C(O)—φ—(CH₂)_r—, where each *r* is independently an integer from 1 to 5 and φ is C₆ aryldiyl or 6-membered heteroaryldiyl and R⁴⁹ is hydrogen, (C₁—C₆) alkyl or a side chain of an encoding or non-encoding amino acid, and where the terminal *sp* carbon is covalently attached to nucleobase B and the other terminal group is covalently attached to R⁴¹.

24. The labeled nucleoside/tide or nucleoside/tide analog of Claim 22 in which nucleobase B is a purine, a 7-deazapurine, a pyrimidine, a normal nucleobase or a common analog of a normal nucleobase.

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25. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 in which D has the structure:



or a salt thereof, wherein:

5

k, *l*, and *m* are each independently integers from 0 to 1;

10 *R*¹, *R*², *R*³, *R*⁴, *R*⁵, *R*⁶ and *R*⁷ are each independently selected from the group consisting of hydrogen, halogen, -F, -Cl, -CN, -CF₃, (C₁-C₆) alkyl, (C₅-C₁₄) aryl or 5-14 membered heteroaryl;

15

MM is a mobility-modifying moiety;

20 *Z* is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹-, where R⁸ and R⁹ when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkylene or (C₄-C₅) alkano;

25

25 *Z'* is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹-, where R⁸ and R⁹, when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkylene or (C₄-C₅) alkano;

30 *R*¹¹, *R*¹², *R*¹³ and *R*¹⁴, when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) arylene, (C₆-C₁₀) arylene independently substituted with one or more W, 6-10 membered heteroarylene and 6-10 membered heteroarylene independently substituted with one or more W;

5 R¹⁹, R²⁰, R²¹ and R²², when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

10 or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) arylene, (C₆-C₁₀) arylene independently substituted with one or more W, 6-10 membered heteroarylene and 6-10 membered heteroarylene independently substituted with one or more W;

15 each W is independently -R, -X, =O, -OR, =S, -SR, -NRR, =NR, (C₁-C₆) perhaloalkyl, -CX₃, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -NHOH, -S(O)₂R, -C(O)R, -C(S)R, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', and -C(NR)NRR, wherein:

20 25 each X is independently a halogen;

each R is independently -H, -NR"R", -C(O)R", -S(O)₂R", (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl;

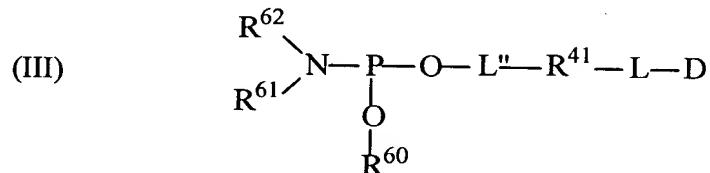
30 each R' is independently (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl and (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each R" is independently -H, (C₁-C₆) alkyl, (C₁-C₆)

alkanyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arlyalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

the dotted line at the heteroaromatic ring nitrogen indicates the point of attachment of linker L.

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26. A mobility-modifying phosphoramidite reagent having the structure:



15 or a salt thereof, wherein:

N, O and P are nitrogen, oxygen and phosphorous, respectively;

D is a mobility-modifying dye chromophore or a protected derivative thereof;

20 L is a first linker;

R⁴¹ is a bond or a covalent linkage;

L" is a bond or a second linker;

R⁶⁰ is a phosphite ester protecting group;

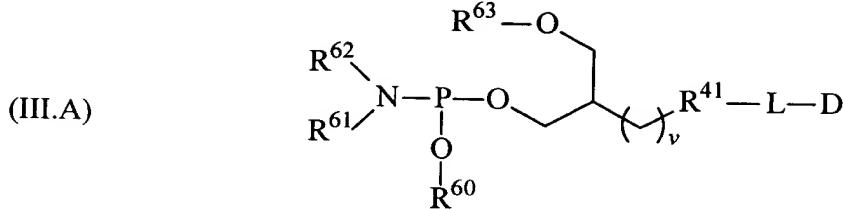
25 R⁶¹, when taken alone, is selected from the group consisting of (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₃-C₁₀) cycloalkyl, (C₅-C₂₀) aryl and (C₆-C₂₆) arylalkyl, or when taken together with R⁶² forms a straight-chain or branched (C₂-C₁₀) alkylene or a straight-chain or branched 2-10 membered heteroalkylene; and

R⁶², when taken alone, is selected from the group consisting of (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₃-C₁₀) cycloalkyl, (C₅-C₂₀) aryl and (C₆-C₂₆) arylalkyl, or when taken together with R⁶¹ forms a straight-chain or branched (C₂-C₁₀) alkylene or a straight-chain or branched 2-10 membered heteroalkylene.

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27. The mobility-modifying phosphoramidite reagent according to Claim 26 which has the structure:



5 wherein:

N, P and O are nitrogen, phosphorous and oxygen, respectively;

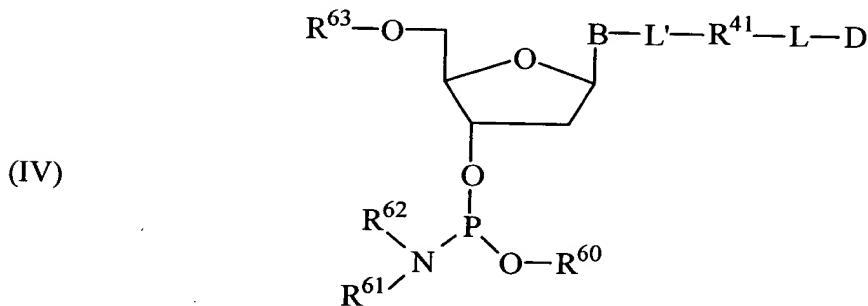
R⁴¹, L, D, R⁶⁰, R⁶¹ and R⁶² are as previously defined;

R⁶³ is hydrogen or an acid-labile hydroxyl protecting group; and

v is an integer from 1 to 30.

10

28. A mobility-modifying phosphoramidite reagent having the structure:



or a salt thereof, wherein:

O, P and N are oxygen, phosphorous and nitrogen, respectively;

20

B is a nucleobase or a protected derivative thereof;

D is a mobility-modifying dye chromophore or a protected derivative thereof;

L is a first linker which is attached to D at a heteroaromatic ring nitrogen;

R⁴¹ is a bond or a covalent linkage;

L' is a bond or a second linker;

R⁶⁰ is a phosphite ester protecting group;

R⁶¹, when taken alone, is selected from the group consisting of (C₁-C₆)

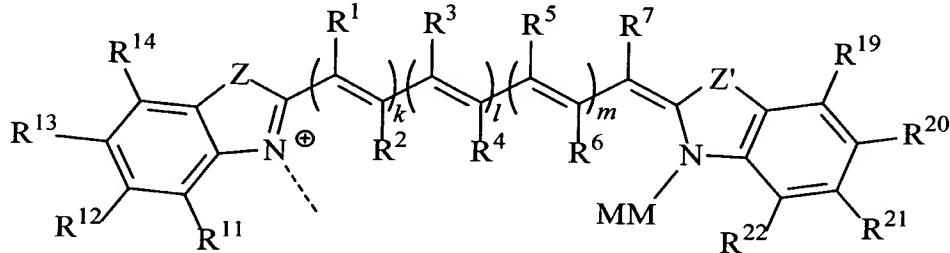
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alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₃-C₁₀) cycloalkyl, (C₅-C₂₀) aryl and (C₆-C₂₆) arylalkyl, or when taken together with R⁶² forms a straight-chain or branched (C₂-C₁₀) alkylene or a straight-chain or branched 2-10 membered heteroalkylene; and

5 R⁶², when taken alone, is selected from the group consisting of (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₃-C₁₀) cycloalkyl, (C₅-C₂₀) aryl and (C₆-C₂₆) arylalkyl, or when taken together with R⁶¹ forms a straight-chain or branched (C₂-C₁₀) alkylene or a straight-chain or branched 2-10 membered heteroalkylene; and

10 R⁶³ is hydrogen or an acid-labile hydroxyl protecting group

29. The mobility-modifying phosphoramidite reagent according to Claim 26, 27 or 28 in which D has the structure:



15 or a salt thereof, wherein:

k, l, and m are each independently integers from 0 to 1;

20 R¹, R², R³, R⁴, R⁵, R⁶ and R⁷ are each independently selected from the group consisting of hydrogen, halogen, -F, -Cl, -CN, -CF₃, (C₁-C₆) alkyl, (C₅-C₁₄) aryl or 5-14 membered heteroaryl;

MM is a mobility-modifying moiety;

Z is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹-, where R⁸ and R⁹ when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkylene or (C₄-C₅) alkano;

25 Z' is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹-, where R⁸ and R⁹, when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkylene or (C₄-C₅) alkano;

R¹¹, R¹², R¹³ and R¹⁴, when taken alone, are each independently selected

from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) aryleno, (C₆-C₁₀) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W;

R¹⁹, R²⁰, R²¹ and R²², when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) aryleno, (C₆-C₁₀) aryleno independently substituted

with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W;

each W is independently -R, -X, =O, -OR, =S, -SR, -NRR, =NR, (C₁-C₆) perhaloalkyl, -CX₃, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -NHOH, -S(O)₂R, -C(O)R, -C(S)R, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', and -C(NR)NRR, wherein:

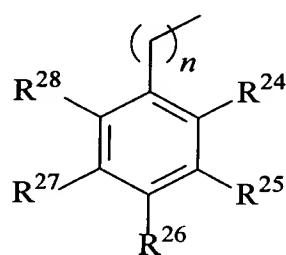
each X is independently a halogen;

each R is independently -H, -NR"R", -C(O)R", -S(O)₂R", (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl;

each R' is independently (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl and (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each R" is independently -H, (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

the dotted line at the heteroaromatic ring nitrogen indicates the point of attachment of linker L.

30. The mobility-modifying phosphoramidite reagent of Claim 29 in which MM has the structure:



or a salt thereof, wherein:

n is an integer from 1 to 6 (preferably 1 to 3);

R²⁴, when taken alone, is hydrogen, a strong anionic substituent, -S(O)₂O⁻,

or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{25} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups;

5 R^{25} , when taken alone, is hydrogen, a strong anionic substituent, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{24} or R^{26} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups;

10 R^{26} , when taken alone, is hydrogen, a strong anionic substituent, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{25} or R^{27} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups; and

15 R^{27} , when taken alone, is hydrogen, a strong anionic substituent, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{26} or R^{28} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups; and

20 R^{28} , when taken alone, is hydrogen, a strong anionic substituent, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{27} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups.

31. The mobility-modifying phosphoramidite reagent according to Claim 26, 27 or 28 in which L is $-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-$ (pentano).

32. The mobility-modifying phosphoramidite reagent according to Claim 26, 27 or 28 in which R^{41} is a covalent linkage formed upon the reaction between an electrophile and a nucleophile.

33. The mobility-modifying phosphoramidite reagent according to Claim 31 in which R^{41} has the structure $-\text{C}(\text{O})-\text{NR}^{56}-$, where R^{56} is hydrogen or (C_1-C_6) alkyl.

30 34. A polynucleotide labeled with a mobility-modifying dye according to

Claim 1.

5 35. A method of generating a labeled primer extension product, comprising the step of enzymatically extending a primer-target hybrid in the presence of a mixture of enzymatically-extendable nucleotides capable of supporting continuous primer extension and a terminator, wherein said primer or said terminator is labeled with a mobility-modifying dye according to Claim 1.

10 36. The method of Claim 35 in which the terminator is a mixture of four different terminators, one which terminates at a template A, one which terminates at a template G, one which terminates at a template C and one which terminates at a template T or U and wherein at least one of the terminators is labeled with a mobility-modifying dye according to Claim 1.

15 37. The method of Claim 36 in which each of the four different terminators is labeled with a different, spectrally-resolvable fluorophore, and one of the terminators is selected from the group consisting of Compound **29** and Compound **32**.

20 38. A kit for generating a labeled primer extension product, comprising enzymatically-extendable nucleotides capable of supporting continuous primer extension and a terminator, wherein said primer or said terminator is labeled with a mobility-modifying cyanine dye according to Claim 1.

25 39. The kit of Claim 38 in which the terminator is a set of four different mobility-modified terminators, one which terminates at a template A, one which terminates at a template G, one which terminates at a template C and one which terminates at a template T or U.

30 40. The kit of Claim 39 in which the set of four different terminators is a set of mobility-matched terminators.

41. The kit of Claim 39 in which the set of mobility-matched terminators comprises Compounds 31, 32, 33 and 34.